## REMARKS

Claims 1-21 were examined and reported in the Office Action. Claims 1-6, 8 and 19 are rejected. Claims 14-15 are canceled. Claims 1-13 and 16-21 are amended. New claims 22-40 are added. Claims 1-13 and 16-40 remain.

Applicant requests reconsideration of the application in view of the following remarks.

### I. 37 CFR §1.83(a)

It is asserted in the Office Action that the drawings are objected to under 37 CFR §1.83(A). Applicant hereby submits new Figure 18 to address the changes recommended by the Patent Office. Figure 18 is based on Applicant's Figure 5 with the addition of microlenses. The support for microlenses can be found, for example, in the description on page 9, lines 33-36: "can be achieved using an array of capillaries or else more perfectly by microlenses." Further, microlenses can be used instead of capillaries. To this end, the microlenses are positioned relative to the capillaries as illustrated in new Figure 18. Thus, the claim limitation of "microlenses" is illustrated. No new matter is added.

The "cell" is illustrated in Figure 1 by the reference numeral 4. The specification refers to "the detection cell 4" on page 6, lines 36, page 7, line 8, page 8, line 20, page 10, lines 25 and 36, page 11, line 4 and page 15, line 1. Thus, the claim limitation of "the cell" is supported in the specification.

The "support" is disclosed in the specification as "consisting of the detection cell wall D" (see page 14, line 34 to page 15, line 1). The detection cell wall D is illustrated on Figure 15 enclosed. Thus, the claim limitation of "the support" is supported in the specification.

Accordingly, Approval of the Patent Office is requested.

II. 37 C.F.R. §1.75(c)

It is asserted in the Office Action that claims 7, 9-18, and 20-21 are objected to under 37 C.F.R. §1.5(c) as being in improper form because a multiple dependent claim cannot serve as the basis for any other multiple dependent claim. See MPEP §608.01(n). Applicant has amended the claims to overcome the 37 C.F.R. §1.5(c) objection.

Accordingly, withdrawal of the 37 C.F.R. §1.75(c) rejection for claims 7, 9-18, and 20-21 are respectfully requested.

III. 35 U.S.C. §112, Second Paragraph

It is asserted in the Office Action that claims 3-6 and 19 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Applicant has amended the claims to overcome the 35 U.S.C. §112, second paragraph rejections.

Accordingly, withdrawal of the 35 U.S.C. §112, second paragraph rejections for claims 3-6 and 19 are respectfully requested.

IV. 35 U.S.C. §102(b)

A. It is asserted in the Office Action that claims 1-3 are rejected under 35 U.S.C. §102(b) as being clearly anticipated by European Patent No. EP723149 issued to Kambara, et al. ("Kambara"). The Applicant respectfully disagrees.

According to MPEP §2131, "'[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.' (Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)). 'The identical invention must be shown in as complete detail as is contained in the ... claim.' (Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989)). The elements must be arranged as required by the claim, but this is not an ipsissimis verbis test, i.e., identity of

terminology is not required. (In re Bond, 910 F.2d 831, 15 USPQ2d 1566 (Fed. Cir. 1990))."

Applicant's amended claim 1 contains the limitations of "... a plurality of juxtaposed capillaries, at least one source for the emission of a beam intended to excite molecules lying in its path and inside the capillaries and means for detecting the fluorescence of the molecules excited by said beam, wherein said means are arranged so as to detect the light which emerges at the exit of said capillaries and which propagates along the direction in which said capillaries extend, the resolution of the detection means is high enough to distinguish the light which emerges at the exit of each of the capillaries, and the refractive index of the media outside of the capillaries is equal or superior to that of the medium inside of the capillaries."

In other words, Applicant's claimed invention relates to electrophoresis systems providing an "on-capillary" excitation (*i.e.*, wherein the source emits a beam intended to excite molecules being located or migrating inside the capillaries). The solution proposed by Applicant's claimed invention is an electrophoresis system wherein total reflection on the fluorescent light inside the walls of the capillaries is avoided. According to Applicant's claimed invention, the detection means only receives fluorescent light that comes directly from the samples and not light that has been reflected several times in the walls or the capillaries. Such a solution makes it possible to detect molecules inside the capillaries while considerably reducing the detection noise coming from the walls.

Kambara discloses an electrophoresis system comprising a plurality of juxtaposed capillaries (1) held by a capillary holder (2), laser sources (21, 22) for the emission of laser beams intended to excite DNA samples (16) and detectors (11a, 11b) for detecting the fluorescence of the samples excited by said beam. Kambara indicates that laser beams generated by laser sources (21, 22) "are applied to the buffer solution path between the capillary holder 2 and the quartz, window 6" (col.7, 1.7-10). Consequently, the sources (21, 22) emit a beam intended to illuminate samples outside the capillaries (see also Fig. 3 where the laser beams do not strike the capillaries). Thus, the system described by Kambara is an electrophoresis system of the type providing a

"post-capillary excitation (*i.e.*, wherein the source emits a beam intended to illuminate samples when located outside the capillary tubes). Note that "Post-capillary" systems are discussed in Applicant's specification as part of the prior art description (see Applicant's specification, page 2, line 30 to page 3, line 16).

Since the system described in Kambara is a "post-capillary" system, the capillaries are not lighted by the light beam emitted by the source, so the detection means does not detect light that emerges at the exit of the capillaries. Moreover, in Kambara, the refractive index of the media outside of the capillaries is not equal or superior to that of the medium inside of the capillaries as recited in claim 1. And, since Kambara relates to electrophoresis systems of the type providing a "post-capillary" excitation, Kambara is not concerned with the problem of detection noise.

Therefore, since Kambara does not disclose, teach or suggest all of Applicant's amended claim 1 limitations, Applicant respectfully asserts that a *prima facie* rejection under 35 U.S.C. §102(b) has not been adequately set forth relative to Kambara. Thus, Applicant's amended claim 1 is not anticipated by Kambara. Additionally, the claims that depend directly or indirectly on claim 1, namely claims 2 -3, are also not anticipated by Kambara for the above same reason.

Accordingly, withdrawal of the 35 U.S.C. §102(b) rejections for claims 1-3 are respectfully requested.

**B.** It is asserted in the Office Action that claims 1-3 are rejected under 35 U.S.C. §102(b) as being clearly anticipated by U.S. Patent No. 5,567,294 issued to Dovichi, et al. ("Dovichi"). The Applicant respectfully disagrees.

Dovichi discloses a multiple capillary analyzer (20) comprising a plurality of juxtaposed capillary tubes (26), a source (130) for the emission of a beam (132) that may excite organic samples and radiation detection means (138) for detecting radiation that is emitted from the samples. The analyzer (20) also comprises a barrier member (90) spaced from the ends (24) of the capillary tubes (26).

In the analyzer (20) described in Dovichi, the source (130) is positioned so as to illuminate the sample streams between the ends (24) of the capillary tubes (26) and the

barrier member (90) (see Dovichi, Figures 2 and 11; column 2, lines56-59; column 5, lines 30-33 and lines 38-39). Consequently, the source (130) emits a beam intended to illuminate samples outside the capillary tubes (26).

Further, Dovichi is an electrophoresis system of the type providing a "post-capillary excitation (*i.e.*, wherein the source emits a beam intended to illuminate samples when located outside the capillary tubes). As the system described in Dovichi are "post-capillary" system, the capillaries are not lighted by the light beam emitted by the source, so the detection means does not detect light which emerges at the exit of the capillaries.

Moreover, in Dovichi, the refractive index of the media outside of the capillaries is not equal or superior to that of the medium inside of the capillaries as recited in claim 1. And, since Dovichi relates to electrophoresis systems of the type providing a "post-capillary" excitation, Dovichi is not concerned with the problem of detection noise.

Therefore, since Dovichi does not disclose, teach or suggest all of Applicant's amended claim 1 limitations, Applicant respectfully asserts that a *prima facie* rejection under 35 U.S.C. §102(b) has not been adequately set forth relative to Dovichi. Thus, Applicant's amended claim 1 is not anticipated by Dovichi. Additionally, the claims that depend directly or indirectly on claim 1, namely claims 2 -3, are also not anticipated by Dovichi for the above same reason.

Accordingly, withdrawal of the 35 U.S.C. §102(b) rejections for claims 1-3 are respectfully requested.

C. It is asserted in the Office Action that claims 1-4 are rejected under 35 U.S.C. §102(b) as being clearly anticipated by Japanese Patent No. JP10019846A issued to Shimadzu ("Shimadzu"). Applicant respectfully disagrees.

Shimadzu discloses a multicapillary electrophoresis apparatus comprising a plurality of juxtaposed capillaries (1a), a source (20) for the emission of a beam (4) intended to excite samples inside the capillaries and a detector (7) for detecting the fluorescence of the samples excited by said beam. The detector (7) is arranged so as to detect the light which emerges at the exit of the capillaries (1a) and which propagates

along the direction in which said capillaries extend. In Shimadzu, the capillary material has a high refractive index relative to the index of the media outside of the capillaries and the medium inside the capillaries in order "to stop the outgoing radiation of the fluorescence from the capillary-tube side" (see Shimadzu, §[0009], lines 4 - 7 of the translation attached). Consequently, in the apparatus of Shimadzu, the fluorescent light emitted by the samples is totally reflected (see Shimadzu, abstract, §"Solution", lines 7-11) by the walls of a capillary and is conducted towards the exit of the capillary as illustrated on Figure 14a enclosed.

On the contrary, according to the limitations of Applicant's amended claim 1, the refractive index of the media outside of the capillaries is equal or superior to that of the medium inside of the capillaries. This not suggested, taught, or disclosed by Shimadzu. Applicant's claimed limitation aims at avoiding total reflection of the fluorescent light on the wall of the capillaries as indicated in the specification at page 13, line 36 to page 14, line 10. The effect of this limitation is illustrated on Figure 13 enclosed: light transmitted through the walls of the capillaries is strongly deflected. This allows discrimination of light transmitted through the walls from light directly emitted by samples. This is not possible with the apparatus disclosed by Shimadzu.

Therefore, since Shimadzu does not disclose, teach or suggest all of Applicant's amended claim 1 limitations, Applicant respectfully asserts that a *prima facie* rejection under 35 U.S.C. §102(b) has not been adequately set forth relative to Shimadzu. Thus, Applicant's amended claim 1 is not anticipated by Shimadzu. Additionally, the claims that depend directly or indirectly on claim 1, namely claims 2-4, are also not anticipated by Shimadzu for the above same reason.

Accordingly, withdrawal of the 35 U.S.C. §102(b) rejections for claims 1-4 are respectfully requested.

## CONCLUSION

In view of the foregoing, it is believed that all claims now pending, namely 1-13 and 16-40, patentably define the subject invention over the prior art of record and are in condition for allowance and such action is earnestly solicited at the earliest possible date.

# PATENT ABSTRACTS OF JAPAN

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G01N 21/91

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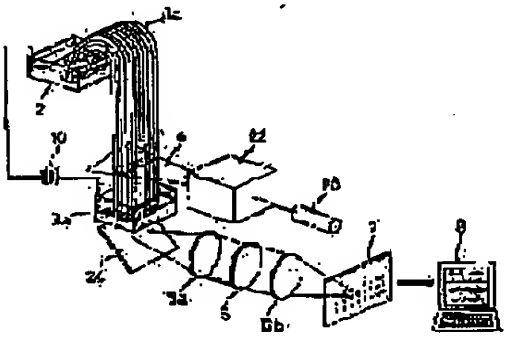
(71)Applicant : SHIMADZU CORP
(72)Inventor : TAKUBO KENJI

## (54) MULTICAPILLARY ELECTROPHORETIC APPARATUS

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain a multicapillary electrophoretic apparatus by which more capillaries can be observed simultaneously.

SOLUTION: A sample which is injected from the upper end of every capillary 1a is migrated downward inside every capillary 1a by a migration voltage which is applied from a power supply 10. During its migration, a linear exciting beam 4 is irradiated, the sample is excited by the laser beam 4 when it is passed through a part irradiated with the laser beam, and fluorescence is emitted. A part of the fluorescence is reflected totally by the surface of every capillary 1a, the fluorescence is not radiated from the side face of every capillary 1a, it is propagated to the length direction of every capillary 1a, and it is radiated from the lower end of every capillary 1a. The fluorescence which is radiated from the lower end of every capillary 1a is reflected by a mirror 24, it is condensed by a lens 5a and a lens 5b, and it is distinguished from background light and exciting light by an optical filter 6 so as to be guided to a detector 7. The output of the detector 7 is input to a computer 8 so as to be processed, and migration waveform data on every capillary 1a is obtained.



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[Number of appeal against examiner's decision of rejection]

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DETAILED DESCRIPTION

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[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] this invention is the electrophoresis apparatus used for biochemical analysis, such as gene diagnosis and DNA sequencing, carries out electrophoresis of the samples, such as a DNA fragment (fragment) pretreated by Sanger's sequencing method using the fluorescence primer (primer to which the chemical bond of the fluorescent substance was carried out as an indicator), and relates to the electrophoresis apparatus of the online formula which detects the fluorescence which is in the middle of migration, irradiates excitation light at a sample and is generated from a sample, and determines a base sequence etc. Especially this invention relates to the multi-capillary-tube electrophoresis apparatus to which electrophoresis of two or more samples is simultaneously carried out using two or more capillary tubes filled up with migration gel.
[0002]
[Description of the Prior Art] The DNA sequencer which is high speed and had a large throughput by high sensitivity is needed for the base sequence determination of DNA with a huge base sequence like a man genome. The multi-capillary-tube DNA sequencer which arranged two or more capillary tubes filled up with gel instead of the thing using plate-like slab gel as the one method is proposed. Compared with slab gel, handling and pouring of a sample make a capillary tube migrate at high speed, and they are not only easy, but can detect it to high sensitivity. That is, if the high voltage is impressed by slab gel, although a band will spread under the influence of the Joule's heat or problems, like a temperature gradient arises will arise, in a capillary tube, there are few such problems, and even if it impresses the high voltage and carries out high-speed migration, there are few breadths of a band and they can perform high sensitivity detection.
[0003] If it processes by Sanger method, four kinds of DNA fragment samples which the end becomes from A (adenine), G (guanine), T (thymine), and C (cytosine) will be generated. The fluorescence formula capillary-tube electrophoresis analysis apparatus which carries out electrophoresis separation of the sample by which the indicator was beforehand carried out by the fluorochrome by the capillary tube as a DNA sequencer, irradiates a laser beam at one point of an electrophoresis way, excites a sample, detects the produced fluorescence, and searches for a migration wave from time change of the intensity is common.
[0004] Drawing 1 shows the example. It fills up with gel in two or more capillary tubes 1 each other arranged in parallel, and the buffer tubs 2 and 3 are arranged at the upper-limit section and soffit section, respectively. In the buffer tub 2 and 3, it is put into buffer liquid. the gel in a capillary tube 1 is contacted. and migration voltage is impressed from the migration power supply 10 between both buffer liquid. It is arranged by the single tier, the excitation light 4 is irradiated from the longitudinal direction of the array, and a capillary tube 1 is detected by the fluorescence detection system arranged in the direction in which the fluorescence which generated the inside of a capillary tube 1 from the sample which migrates intersects the array side of a capillary tube 1. A fluorescence detection system is equipped with a single dimension or the 2-dimensional picture detector 7, the optical system 5a and 5b for carrying out image formation of the image on one line irradiated by excitation light on the picture detector 7, and the filter 6 from which fluorescence is made to penetrate among the light from the sample in a capillary tube, and an excitation light component is removed, and detects the fluorescence image from the capillary tube by which image formation was carried out to the detector 7. The base sequence of DNA etc. is determined from time change of the fluorescence detected for every capillary tube.
[0005]
[Problem(s) to be Solved by the Invention] In the multi-capillary-tube electrophoresis apparatus of drawing 1, only the capillary tube arranged at the single tier can be observed, but the number of the samples which can be analyzed simultaneously is restricted. Though a 2-dimensional picture detector is used as a detector 7, only the picture of the single dimension of them can be used.

[0006] Moreover, when incidence of the excitation light is carried out from the longitudinal direction of the array of a capillary tube, excitation light intensity differs greatly by the incidence and outgoing radiation side, a difference will arise in fluorescence intensity and a S/N ratio will change with the positions of a capillary tube. this invention aims also at preventing excitation light intensity differing, arranging many capillary tubes more, and enabling it to observe simultaneously by the place where the capillary tube is arranged.

[0007]

[Means for Solving the Problem] The base or the side which the buffer tub prepared in the soffit section of a capillary tube in the multi-capillary-tube array migration section by which is equipped with two or more capillary tubes which it filled up with gel and were arranged in parallel, and electrophoresis is simultaneously carried out by all capillary tubes counters the soffit side of a capillary tube is constituted from the transparent member by this invention. From the direction which intersects the field of a capillary-tube array, excitation optical system irradiates an excitation light beam along with one straight line which intersects perpendicularly with the migration direction at all capillary tubes. Moreover, a fluorescence detection system is equipped with the optical system which leads to the detector what penetrated and carried out outgoing radiation of the transparent base or the transparent side of a buffer tub of the soffit section from the soffit side of a capillary tube by the fluorescence generated from the sample of all the capillary tubes excited by the detector and the excitation light beam of excitation optical system.

[0008] In this invention, since it was made to detect fluorescence from the soffit side of a capillary tube, a capillary-tube array can also be arranged to multiplex and the number of the samples which can be analyzed simultaneously can be increased sharply.

[0009]

[Example] Drawing 2 expresses one example. Mutually, in parallel, capillary-tube 1a which is a migration way is arranged so that an end face may be arranged at two dimensions. Although it fills up with the gel of migration support in capillary-tube 1a, since capillary-tube 1a and gel do not attenuate fluorescence, the thing of the transparent quality of the material is desirable. Moreover, in order to stop the outgoing radiation of the fluorescence from the capillary-tube side, the quality of the material of capillary-tube 1a has the desirable thing of a high refractive index to air and buffer liquid. As the quality of the material of such capillary-tube 1a, quartz glass is suitable. Moreover, as gel, a polyacrylamide gel is suitable.

[0010] The upper limit of the bunch of capillary-tube 1a is dipped in the buffer liquid in the upper-limit side buffer tub 2, and the soffit of the bunch of capillary-tube 1a is dipped in the buffer liquid in soffit side buffer tub 3a. The base of soffit side buffer tub 3a which counters the soffit side of capillary-tube 1a consists of the transparent quality of the materials.

[0011] Since the excitation light beam 4 is generated, the Ar ion laser and the YAG laser are prepared as the light source 20, and an Ar ion laser, an YAG laser, and optical system are arranged so that the laser beam from the laser beam and YAG laser from an Ar ion laser may serve as an excitation light beam on the same optical axis. One method of generating the excitation light beam 4 oscillates a 488nm laser beam from an Ar ion laser, and makes the laser beam which is made to oscillate a 532nm laser beam and contains two kinds of wavelength the excitation light beam 4 from an YAG laser. Only using an Ar ion laser, other methods of generating the excitation light beam 4 oscillate simultaneously the laser beam of two kinds of the wavelength (488nm and 514.5nm), and let them be the excitation light beams 4.

[0012] In order to make the excitation light beam 4 into the thing of the shape of a straight line perpendicular to the migration direction, a beam expander and the optical system 22 containing the cylindrical lens are arranged on the optical path of the excitation light beam 4. A beam expander extends a beam, extends the excitation light beam which carries out incidence from the light source 20, and irradiates in the direction of a capillary-tube array. The excitation light beam 4 which a cylindrical lens makes converge the excitation light beam 4 which was able to be extended by the beam expander in the shape of a line, and it converged in the shape of a line is irradiated by the capillary-tube array along with the straight line which intersects perpendicularly with the migration direction from the direction which intersects the field of a capillary-tube array. The light source 20 and optical system 22 constitute excitation optical system.

[0013] In order to lead the light which carried out outgoing radiation from the soffit side of capillary-tube 1a to the 2-dimensional detector 7, a mirror 24 is arranged on the outside of the base of buffer tub 3a, and in order to carry out image formation of the light reflected by the mirror 24 to a detector 7, Lenses 5a and 5b are arranged. Among Lenses 5a and 5b, the light filter 6 for making only a fluorescence component penetrate among the light which carried out outgoing radiation from the end face of a capillary tube is arranged. As a detector 7, a CCD camera, a vidicon (image pick-up tube), etc. are suitable.

[0014] Operation of this example is explained. An indicator is carried out with a fluorescent substance and the sample poured in from the upper limit of capillary-tube 1a migrates the inside of capillary-tube 1a below with the migration voltage impressed from a power supply 10. If the line-like excitation light 4 is irradiated in the

middle of migration and a sample passes the irradiation part of the laser beam 4, it will be excited by the laser beam and fluorescence will be emitted. A part of this fluorescence is spread in the length direction of capillary-tube 1a, without carrying out total reflection on the front face of capillary-tube 1a, and carrying out outgoing radiation from the side of capillary-tube 1a, and it carries out outgoing radiation from the soffit of capillary-tube 1a. In order to suppress dispersion in a capillary-tube 1a front face at this time, it is desirable to use a capillary tube with a smooth front face, and to carry out short **** composition of the distance from the laser radiation part of a capillary tube to a soffit as much as possible. It is reflected by the mirror 24 and condensed with Lenses 5a and 5b, and after the light which carried out outgoing radiation from the soffit of capillary-tube 1a is distinguished from background light and excitation light with a light filter 6, it is led to a detector 7. The output of a detector 7 is inputted into a computer 8, and is processed, and the migration data point of each capillary-tube 1a is obtained.

[0015]

[Effect of the Invention] In this invention, since it was made to detect fluorescence from the soffit side of a capillary tube, a capillary-tube array can also be arranged to multiplex and the number of the samples which can be analyzed simultaneously can be increased sharply. Moreover, since the fluorescence which carries out outgoing radiation from the end face of a capillary tube is detected, incidence to a fluorescence detection system can be made easy by bending a capillary tube.

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## CLAIMS

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[Claim(s)]
[Claim 1] The multi-capillary-tube electrophoresis apparatus characterized by providing the following. It is prepared in the upper-limit section and the soffit section of two or more capillary tubes and a capillary tube which it filled up with gel and were arranged in parallel mutually. The buffer tub by which the base or the side which counters the soffit side of a capillary tube by the soffit section side while holding the buffer liquid which contacts the gel in a capillary tube electrically is constituted from a transparent member, And the multi-capillary-tube array migration section by which it has the migration power supply which impresses migration voltage to the gel in a capillary tube through both buffer liquid, the sample by which the indicator was carried out with the fluorescent substance is injected into each capillary tube, and electrophoresis is simultaneously carried out by all capillary tubes. Excitation optical system which irradiates an excitation light beam along with one straight line which intersects perpendicularly with the migration direction at all capillary tubes from the direction which intersects the field of the capillary-tube array of the aforementioned multi-capillary-tube array migration section. A detector and the fluorescence detection system equipped with the optical system which leads to the aforementioned detector what penetrated and carried out outgoing radiation of the transparent base or the transparent side of a buffer tub of the soffit section from the soffit side of a capillary tube by the fluorescence generated from the sample of all the capillary tubes excited by the excitation light beam of the aforementioned excitation optical system.

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DESCRIPTION OF DRAWINGS

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[Brief Description of the Drawings]
[Drawing 1] It is the outline perspective diagram showing the conventional electrophoresis apparatus.
[Drawing 2] It is the outline perspective diagram showing the electrophoresis apparatus of one example.
[Description of Notations]
1a Capillary tube
2 3a Buffer tub
4 Excitation Light Beam
5a, 5b Lens
7 2-dimensional Detector
20 Light Source
22 Optical System

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DRAWINGS

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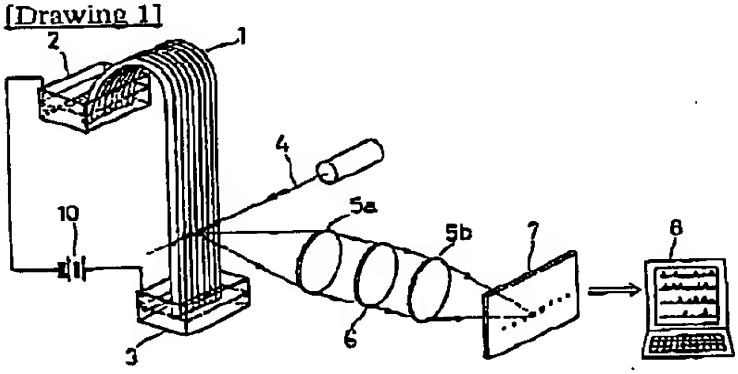
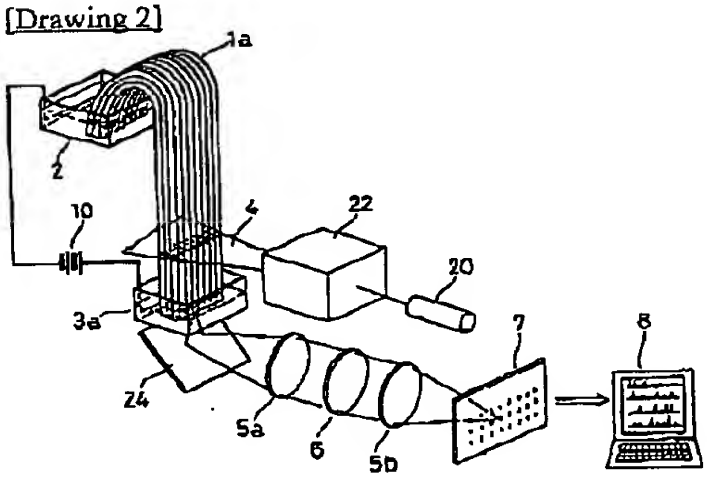
[Drawing 1]




[Drawing 2]

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